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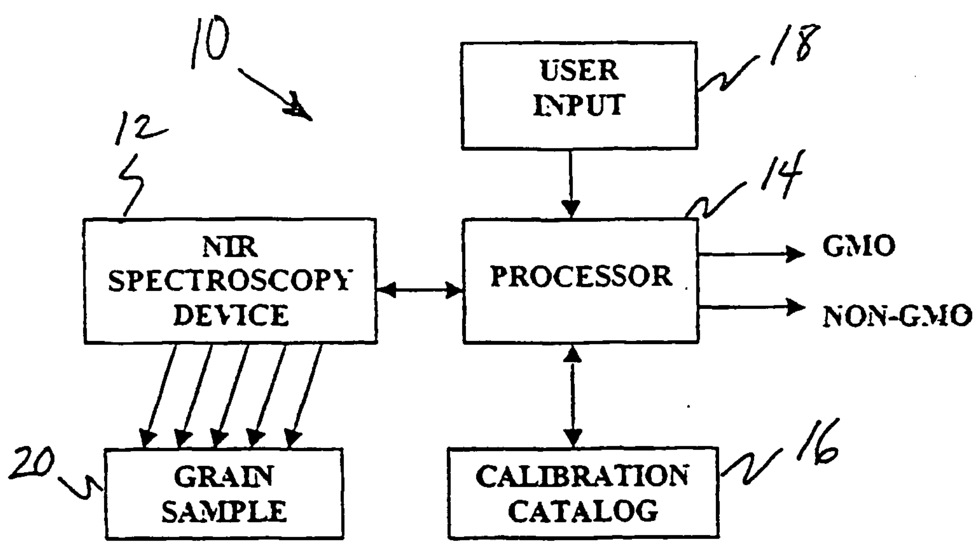
(54) Title: NEAR INFRARED SPECTROSCOPY SYSTEM AND METHOD FOR THE IDENTIFICATION OF GENETICALLY MODIFIED GRAIN



(57) Abstract: Genetically modified (GMO) grain can be distinguished from non-GMO grain by a test procedure that makes use of near infrared (NIR) spectroscopy. Calibrations derived for individual GMO events enable positive identification of GMO and non-GMO grains. The procedure is particularly useful at grain handling or delivery points, such as local elevators. System software and hardware can be incorporated in the grain stream to permit analysis of intermittent grain samples. In many cases, the necessary calibrations can be implemented in existing NIR instrumentation.

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NEAR INFRARED SPECTROSCOPY SYSTEM AND METHOD FOR THE IDENTIFICATION OF GENETICALLY MODIFIED GRAIN

TECHNICAL FIELD

The present invention relates to analysis of grain and, more particularly, to identification of genetically modified grain.

BACKGROUND

Identification of genetically modified ("GMO") grain products has become an important issue in agriculture for producers, grain processors and grain exporters. For example, some new crops are genetically modified to produce grain with a nutrient composition specially designed to benefit a particular animal species, such as chickens or pigs. Such grain can command a premium price, provided that its identity is preserved through the chain of commerce, from the producer to the end user. In addition, some countries have proposed labeling of foods containing genetically modified grain, which may require grain handlers to distinguish between GMO and non-GMO grain.

Simple verification of labels and purchase receipts may not be sufficient to ensure the identity of a particular lot of grain as GMO or non-GMO grain. There is a possibility that non-GMO seed may be inadvertently mixed with non-GMO seed, or vice versa. The problem is complicated by the fact that firms buying the grain at original point of delivery locations, such as local grain elevators, ordinarily are not equipped to identify grain as GMO or non-GMO on site. Therefore, grain purchasers and processors may seek to impose warranty conditions on grain producers.

Wet chemical tests for GMO and non-GMO grain identification are presently available, and are reasonably effective. The tests are technically complicated, however, and can require an extended period of time to obtain the results. Consequently, use of wet chemical technologies for on-site verification at locations such as elevators is generally not feasible.

SUMMARY

The present invention is directed to methods and systems for distinguishing between genetically modified (GMO) and non-GMO grains using near infrared (NIR)

spectroscopy, either reflectance, absorbance or transmittance. NIR spectroscopy can be used to differentiate GMO from non-GMO grain with a very high degree of accuracy upon calculation of NIR calibrations for individual GMO events. In this manner, NIR spectroscopy can be used to quickly verify the presence or absence of GMO grain.

Verification using NIR spectroscopy can reduce the difficulties associated with identity preservation of grain for seed companies, grain producers, grain processors, grain exporters and purchasers. Moreover, NIR spectroscopy requires little technical expertise and can provide results in a shorter period of time, compared to wet chemistry techniques.

NIR spectroscopy can be applied to analyze samples taken from a grain stream. Alternatively, individual samples can be taken or submitted manually and subjected to NIR spectroscopy. In either case, the use of NIR spectroscopy provides a rapid and accurate technique for identification of GMO grain, with little impact on grain throughput.

The NIR spectroscopy process can be automated, and can be implemented in part by adaptation of existing NIR spectroscopy equipment already used in many grain handling locations. Accordingly, this technique can be especially advantageous for high-throughput facilities, such as seed company production facilities or local grain elevators, where prompt verification, minimal cost, and limited technical intervention are desirable.

The NIR spectroscopy technique can be particularly useful for identification of GMO and non-GMO soybeans and corn. Other types of grains, amenable to differentiation between GMO and non-GMO grains, include small grains such as barley, oats, wheat, rye and rice. It is contemplated that NIR calibrations can be calculated for so-called "stacked" GMO events in grains, providing an indication for each event.

Users may dispense with lengthy, complicated wet chemistry techniques in favor of the more rapid results provided by the simple NIR spectroscopy technique. Indeed, the NIR spectroscopy device can be coupled to a computer that processes the data and generates a discrete positive or negative indication concerning the presence or absence of GMO grain. Users can make use of a catalog of calibrations between

GMO and non-GMO products, which take into account differences in spectral signature between grain type, GMO event, and geographic regions. The catalog can be organized to allow selection of the desired calibrations by the user. The catalog can be stored by a computer associated with the NIR spectroscopy device, which permits calibrations to be selectively retrieved for use with spectral data from individual grain samples.

A different calibration ordinarily will be provided for each known GMO event and grain type. Accordingly, as new GMO grains are added to the market, the catalog can be updated, e.g., by shipment on physical media or downloading via the internet. Users may elect to obtain the entire catalog and subscribe to updates. Alternatively, a user may elect to obtain specific portions of the catalog. In this manner, a user can be provided with only those calibrations best suited for grain produced or processed at each location.

In one embodiment, the present invention provides a method for distinguishing between genetically modified (GMO) and non-GMO grain, the method comprising subjecting a grain sample to near infrared spectroscopy to produce spectral data, and determining whether the grain sample contains GMO grain based on the spectral data.

In another embodiment, the present invention provides a system for distinguishing between genetically modified (GMO) and non-GMO grain, the system comprising a near infrared spectroscopy device that subjects a grain sample to near infrared spectroscopy to produce spectral data, and a processor that determines whether the grain sample contains GMO grain based on the spectral data produced by the near infrared spectroscopy device.

In another embodiment, the present invention provides a method for distinguishing between genetically modified (GMO) and non-GMO grain, the method comprising measuring the infrared spectral signature of a grain sample, and determining whether the grain sample contains GMO grain based on the analysis.

In a further embodiment, the present invention provides a method for identifying genetically modified (GMO) grain, the method comprising analyzing a grain sample without application of wet chemical assay, and determining whether the grain sample contains GMO grain based on the analysis.

In another embodiment, the present invention provides a method for identifying genetically modified (GMO) soybeans, the method comprising subjecting a sample of soybean seed to near infrared spectroscopy to produce spectral data, and determining whether the sample contains GMO soybeans based on the spectral data.

Other advantages, features, and embodiments of the present invention will become apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of a system for identification of GMO grain;

FIG. 2 is a flow diagram of a calibration and measurement process for GMO grain identification;

FIG. 3 is a graph illustrating NIR spectral data for GMO and non-GMO grain samples;

FIG. 4 is a histogram of calibration values for a first NIR spectroscopy device; and

FIG. 5 is a histogram of calibration values for a second NIR spectroscopy device.

Like reference numbers and designations in the various drawings indicate like elements.

DETAILED DESCRIPTION

FIG. 1 is a block diagram of a system 10 for identification of GMO grain. As shown in FIG. 1, system 10 includes an NIR spectroscopy device 12, a processor 14, a storage device 16, and a user input device 18. Processor 14 controls device 12 to subject a grain sample 20 to near infrared spectroscopy. Device 12 collects NIR spectral data from grain sample 20 and transmits the data to processor 14. Processor 14 determines whether the grain sample contains GMO grain based on the spectral data. Storage device 16 stores a catalog containing a plurality of calibration equations indicative of differences in spectral characteristics for particular GMO and non-GMO grains. Processor 14 applies the spectral data to a calibration equation retrieved from storage device 16 to calculate a value. The calculated value indicates whether grain sample 20 contains grain with a GMO event.

Processor 14 can be realized by a general purpose computer, such as a personal computer or workstation, that executes program code to carry out the NIR spectroscopy process in combination with device 12. Alternatively, processor 14 can be integrated with device 12. Storage device 16, which stores a catalog of calibration equations, can be realized by a hard drive or removable media drive associated with the computer. In addition to code arranged to drive the spectroscopy process, processor 14 may execute statistical, analysis applications for generation of the calibration equations and application of spectral data to the calibration equations to identify GMO grain in grain sample 20. A commercially available software application suitable for analyzing spectral data generated by NIR device 12 is the Unscrambler™ software marketed by Camo A/S, of Oslo, Norway. User input device 18 can be used by a user to select particular calibration equations or categories thereof, and activate the spectroscopy process. Alternatively, the spectroscopy process can be automated.

NIR device 12 can take the form of a commercially available NIR device, such as the Foss Grainspec™ or Foss/Tecator Infratec™ NIR instruments, available from Foss North America, of Eden Prairie, Minnesota, USA. Both instruments are presently used in a number of grain elevators throughout the Midwest United States to detect a variety of grain characteristics such as moisture, protein, oil, and fiber content. Both instruments operate in reflectance mode. With appropriate calibration equations, GMO grain identification can be implemented using existing NIR instruments, including those already installed at grain elevators.

New calibrations can be loaded into NIR device 12 or a memory coupled to an associated processor 14, and used to identify GMO grains. A separate calibration is provided for each GMO event in each type of grain and for each type or model of NIR device 12. With adequate computing capacity in processor 14, all available calibrations for a product can be calculated almost simultaneously, providing quick feedback to a user concerning the genetic makeup of a grain sample and the presence of multiple GMO events.

Typical GMO grains are obtained by introduction into of plant of a preselected DNA segment, e.g., an exogenous or recombinant DNA sequence. The DNA is introduced into the genome of the plant by transformation. Each successful

transformation event (GMO event) introduces a gene(s) for a desired plant attribute. Useful GMO events include, for example, genes conferring tolerance to herbicides such as glyphosate (Roundup Ready™), bromoxynil, sulfonylureas or phosphinothricin resistance genes, genes conferring insect resistance such as *Bacillus thuringiensis* endotoxin protein genes and genes for specialty traits such as improved amino acid composition, e.g., dihydrodipicolinic acid synthase genes, 10kD zein genes, 15kD zein genes, aspartate kinase genes, or soybean albumin seed protein genes, as well as antisense or ribozyme genes that inhibit expression of an endogenous gene.

Device 12 can be realized by an NIR instrument commonly used in local grain elevators to collect spectral data in the 850-1050 nm range to detect the characteristics of the samples. If device 12 is a Foss Grainspec™, for example, spectral values are collected at 33 wavelengths in the 850-1050 nm range. If device 12 is a Foss/Tecator Infratec™, 100 spectral values in the same wavelength range are collected. Other NIR devices, which collect reflectance data in the mid-infrared spectral ranges (about 600-2500 nm) can also be used.

NIR devices are currently used to analyze grain for, e.g., moisture, protein, oil, fiber, and saturated fats, and typically complete the analysis in approximately 60 seconds per sample. A wide range of samples is collected each harvest with distinct compositional values to make a range for the different calibrations. The calibrations, a set of multiple linear equations with spectral values, in Beer's Law form ln(1/T), as the independent variable, are statistically based upon the reference values of the samples collected.

For system 10 to detect the difference between the GMO and non-GMO grains, the two types of grains must have a structural or biochemical distinction that would affect NIR spectral properties. The existence of such differences was not previously known. Indeed, previous studies of genetically modified grains have indicated that there are structural and biochemical similarities between GMO and non-GMO grains.

FIG. 2 is a flow diagram of a calibration and measurement process for GMO grain identification. As shown in FIG. 2, the calibration process, indicated generally by block 22, involves obtaining a representative product sample set, as indicated by

block 24. The sample set may include, for example, one hundred or more different samples of a particular grain type, each sample known to be GMO or non-GMO grain. Each sample is subjected to NIR spectroscopy to collect spectral data, as indicated by block 26. The spectral data are typically collected at a plurality of different wavelengths. As indicated by block 28, the spectral data also can be collected at a number of different sample temperatures to permit identification of temperature-induced variations in the spectral signature. Reference values are then assigned to each sample such that a GMO event is a value of 1, whereas a non-GMO event is a value of 0, as indicated by block 30. Processor 14, for example, can be appropriately programmed to carry out the desired assignment of a reference value.

Next, the spectral data are subjected to pretreatment and statistical analysis, as indicated by block 32. As indicated by block 34, for example, the spectral data can be smoothed, normalized, and filtered prior to application of a statistical analysis technique such as partial least squares (PLS), multiple linear regression (MLR), or neural network (NN) analysis. Such pretreatment differs from known NIR spectral data pretreatments. The usual objective of NIR calibrations is to make the calibrations insensitive to shifts in spectral data across the entire wavelength range. This permits the usual calibrations to identify relative differences in spectra between a reference sample and a test sample that result from quantitative differences in the chemical constituents of the grain (e.g., protein, oil, starch, individual amino acids and the like). In contrast, the pretreatments applied herein include the predicted constituent values of bias-insensitive calibrations in the independent variables. This type of pretreatment allows the statistical model to identify average spectral differences attributable to the GMO event from those attributable to constituents alone.

Although unlike usual NIR pretreatments, the calibrations described herein for detecting GMO and non-GMO grain can be readily implemented in software. Each calibration equation may correspond to one GMO event and may take the form of a multi-term equation, as indicated by block 38. The statistical software calculates the best fit for the wavelength information to the reference value, i.e., discrete values of 1 or 0. An equation is produced with as many terms as independent variables, often in the form $y = b0+b1X1+ \ldots bnXn$, where b values are generated by the calibration software. The linear form is not necessary, however, and the inclusion of the

previously predicted constituent levels generally tends to make the calibration equations non-linear.

Calibration equations can be prepared and generated at various local sites, if desired. It ordinarily will be more desirable, however, to generate the calibration equations at a central laboratory and then distribute them to users in the field for installation, as indicated by block 40. Calibration equations can be distributed to a virtually unlimited number of NIR systems 10. For example, distribution can be by physical media such as CD-ROM, DVD-ROM and tapes, or by electronic downloads via dial-up or internet connections. In either case, the calibration equations can be widely disseminated, particularly to local grain elevators, and updated from time-to-time when new GMO varieties are being grown in a region. Although a large number of calibration equations may be distributed, a user can select an appropriate category or categories of equations based on the type of grain, the type of GMO event, or the region from which the grain was produced.

Once the calibration equations are loaded into the NIR system, the measurement process, generally indicated by block 44, can be applied to identify the presence of GMO grains. As indicated by block 46, it may be desirable to standardize individual NIR devices 12 by analyzing a number of samples having known spectral signatures, e.g., ten to twenty samples, both in the instruments to be used at other locations and in a master unit (the unit that was used to create the calibrations). There are generally small differences in the optical properties of individual instruments that must be accounted for if all instruments are to most effectively utilize the same calibration equations. Preferably, standardization is accomplished by applying a bias to the values calculated for the known samples by an individual unit. Such a bias forces the values calculated by an individual unit to correspond to those obtained on a master unit, and can be achieved in software or hardware. Alternatively, spectral data obtained from the spectroscopy device 12 can be modified in software or hardware to standardize the data to spectral data from the master unit, prior to use of the calibration in the GMO grain analysis process. As another alternative, procedures can be used to adjust, wavelength by wavelength, the spectral data of all individual units before the calibration equation is calculated. It is contemplated that any of the above approaches will achieve satisfactory GMO calibrations. However, the bias approach, which is in effect shifting the rounding point up or down from an initial value of 0.5,

is expected to be the simplest and most adaptable method, since calibrations for the presence or absence of GMO grain require a discrete, qualitative classification.

Following standardization, spectral data is collected, as indicated by block 48, for the desired grain samples, indicated by block 50. The grain samples can be manually prepared and placed within the NIR device by known techniques. Alternatively, the measurement process can be readily automated, e.g., using a conveyor that intermittently removes grain samples from a grain stream, prepares them for analysis and delivers them to the NIR device. Upon collection of spectral data, the processor associated with the NIR device computes event calibration values using the calibration equations, as indicated by block 52. The processor may compute the event calibration values using any number of calibrations, as indicated by block 54. In this manner, a sample can be tested against calibrations for multiple GMO events, providing an indication of the presence or absence of each event to the user.

With reference values of 1 and 0, the calibration values can provide a simple "yes" or "no" indication of the presence of a GMO event, as indicated by blocks 56 and 58. By rounding values to 1 or 0, the value is always an integer, as indicated by block 60. In the embodiment of FIG. 2, if the calibration value is greater than or equal to 1, the sample is classified as a GMO grain of the respective event, as indicated by block 62. If the calibration is less than or equal to 0, the grain sample is classified as a non-GMO grain for the respective event, as indicated by block 64. The values could, of course, be reversed, i.e., 1 could be indicative of a non-GMO grain and 0 could be indicative of a GMO event. The objective is to formulate the calibration equations to produce, from the spectral data, a simple indication of the presence or absence of a GMO event. In this manner, the output of the measurement process can be a discrete "yes" or "no," or positive or negative result to be received by the user. An indicator device such as a display, status light, or audible alarm can be coupled to the processor to facilitate notification of a user. With multiple calibration equations, however, it should be noted that the user may receive more than one "yes" or "no" indications if multiple GMO event grain is analysed. Raw measurement values can also be displayed on the indicator device, if so desired by a user.

EXAMPLE

The following example illustrates the application of a GMO grain identification technique in accordance with an embodiment of the present invention. Experiments were conducted using Foss Grainspec™ and Foss/Tecator Infratec™ NIR instruments, available from Foss North America, of Eden Prairie, Minnesota, USA. The spectral data were analyzed with Unscrambler™ software, available from Camo A/S, of Oslo, Norway.

Grain samples were collected from strip-plots located in Bremer and Tama/Grundy counties in Iowa, USA, that contained soybeans known to be either GMO or non-GMO. The GMO soybeans contained a gene conferring tolerance to glyphosate (Roundup™), and are referred to herein as RR soybeans. The non-GMO soybeans did not contain the glyphosate tolerance gene or any other transgene. Of the non-RR soybeans, 35 samples came from the Bremer county plot and 28 came from the Tama/Grundy county plot. Of the RR soybeans, 24 samples came from the Bremer county plot and 27 came from the Tama/Grundy county plot.

Samples from each plot were divided into non-RR and RR categories. The samples were prepared run in the Infratec and Grainspec devices successively. The NIR data were collected, along with estimated protein, oil, fiber, and saturated fat percentages, calculated from existing calibrations. The Grainspec device collects optical values at 33 wavelengths in the 850-1050 nm range. The Infratec device collects 100 values in the same wavelength range. The calibration and subsequent user-operated measurement process is illustrated in the block diagram of FIG. 2.

The composition results showed a very strong correlation between the two instruments, as indicated in Table 1 below, and little compositional difference between the two types of soybeans. The nutritional composition of the samples was similar to that of the overall 1998 soybean harvest in Iowa.

TABLE 1

	GrainSpec					Infratec			
	Moisture (%)	Protein (%)	Oil (%)	Fiber (%)		Moisture (%)	Protein (%)	Oil (%)	Fiber (%)
Overall GrainSpec					Overall Infratec				
Avg	11.4	36.3	18.7	4.7	Avg	11.4	36.0	18.9	4.9
Max	13.5	37.8	20.1	5.0	Max	13.1	37.7	20.0	5.1
Min	9.3	34.3	17.5	4.2	Min	9.5	34.0	17.9	4.7
Std Dev	0.63	0.76	0.49	0.14	Std Dev	0.56	0.77	0.44	0.09
Count	115				Count	114			
Non-GMO Samples					Non-GMO Samples				
Avg	11.2	36.3	18.6	4.6	Avg	11.2	36.0	18.9	4.9
Max	12.7	37.8	19.8	5.0	Max	12.6	37.7	19.7	5.1
Min	9.3	34.8	17.5	4.2	Min	9.5	34.4	18.0	4.7
Std Dev	0.69	0.74	0.49	0.15	Std Dev	0.63	0.69	0.43	0.10
Roundup Ready™ Samples					Roundup Ready™ Samples				
Avg	11.6	36.1	18.7	4.7	Avg	11.6	36.0	18.9	4.9
Max	13.5	37.1	20.1	4.8	Max	13.1	37.5	20.0	5.1
Min	10.9	34.3	17.8	4.3	Min	11.0	34.0	17.9	4.7
Std Dev	0.45	0.77	0.50	0.11	Std Dev	0.37	0.86	0.45	0.09

a Basis 13% moisture

1998 Iowa averages from the ISU annual national soybean quality survey were 35.8% protein, 19.1% oil, and 4.9% fiber.

FIG. 3 is a graph illustrating NIR spectral data for GMO and non-GMO grain samples. In particular, the graph of FIG. 3 shows the difference in average spectral data between RR and non-RR samples. Spectral data for non-RR samples is indicated by reference numeral 66, whereas spectral data for RR samples is indicated by reference numeral 68. The nearly constant difference means that the constituent calibration could be use to model this, but also that a specific regression intended to find differences would also be successful.

For RR identification, a reference value of zero was input for the non-RR soybeans and a reference value of one was input for the RR soybeans. The ones and zeros replaced chemistry values and were then regressed against the spectral values. Through this process, an equation was derived for each instrument. This equation gave a score for each sample. A histogram of the scores can be found in FIG. 4 (Grainspec) and FIG. 5 (Infratec). The shape of the histogram shows that the Grainspec had a slight advantage at separating the classes. The two-peak shape in FIG. 4 reflects concentration around two values, 1.0 and 0.0. Inspection of the histogram in FIG. 5 suggested that a lower cutoff value (about 0.4) may be more suitable for classifying grain when using the Infratec instrument.

Samples lying on the extreme outer limits of the set (optically) were considered outliers and were not used in the determination of the line. Samples were outliers for a number of reasons. There were several damaged or incomplete scans. Samples were also at the optical extremes to the point where they could not be considered statistically part of the group. If the sample is on the outer edge of a grouping, it is considered not part of the family of centralized samples, which causes it to be recognized as an outlier.

If the computed result for a non-RR sample was rounded to one or greater, the sample was identified incorrectly. If the computed result for a RR sample was rounded to zero or less, the sample was identified incorrectly. Overall, the Grainspec and Infratec test instruments correctly identified 99.0% and 89.7% of the samples, respectively, as indicated by Table 2 below. The Grainspec device correctly identified a somewhat higher percentage, and both instruments did better at identifying non-RR than RR. A larger database would likely improve accuracy of both instruments.

TABLE 2

	Number of Samples	Number of Outliers	Correctly Classified	Classified as Other	Percent Correctly Identified
Bremer County Soybeans					
GrainSpec non-Roundup Ready	35	5	30	0	100.0
GrainSpec Roundup Ready	24	3	21	0	100.0
Infratec non-Roundup Ready	35	5	30	0	100.0
Infratec Roundup Ready	24	2	20	2	91.1
Tama/Grundy County Soybeans					
GrainSpec non-Roundup Ready	28	3	24	1	96.0
GrainSpec Roundup-Ready	27	3	24	0	100.0
Infratec non-Roundup Ready	28	6	17	5	77.3
Infratec Roundup Ready	27	4	20	3	87.1
Overall Results					
GrainSpec	114	14	99	1	99.0
Infratec	114	17	87	10	89.7

The Infratec calibration was used to test 39 additional soybean samples, 20 non-RR samples from Linn County, Iowa, and 19 RR samples from Clay County, Iowa. The results are shown in Table 3 below. With a -0.1 bias, 95% of the non-RR and 84% of the RR samples were identified correctly. The bias meant that the cutoff between non-RR and RR classification, i.e., the point at which there is the least overlap between types, was 0.4 instead of 0.5. The histogram of FIG. 5 had suggested that a lower cutoff value might be preferable. There were no outliers in the validation set. The bias improved the accuracy of identifying RR, but did not affect identification of non-RR soybeans. These results show that calibration equations could be developed to accurately distinguish between RR and non-RR soybeans.

TABLE 3

Class	n	Bias Applied[a]	Correctly Classified	Percent Correctly Classified
Non-Roundup Ready	20	0.0	19	95.0
		-0.1	19	95.0
Roundup Ready™	19	0.0	11	57.9
		-0.1	16	84.2

[a]Change to the value used as cutoff between non-Roundup Ready and Roundup Ready soybeans (0.0 = 0.5 cutoff; -0.1 = 0.4 cutoff)

The foregoing detailed description has been provided for a better understanding of the invention and is for exemplary purposes only. Modifications may be apparent to those skilled in the art without deviating from the spirit and scope of the appended claims.

CLAIMS:

1. A method for distinguishing between genetically modified (GMO) grain and non-GMO grain, the method comprising:

subjecting a grain sample to near infrared spectroscopy to produce spectral data; and

determining whether the grain sample contains GMO grain or non-GMO grain based on the spectral data.

2. The method of claim 1, wherein the determining step comprises applying the spectral data to a calibration equation that defines a spectroscopic difference between a non-GMO grain and a GMO grain to calculate a value that indicates whether the sample contains GMO grain or non-GMO grain.

3. The method of claim 2, further comprising providing an indication of the calculated value to a user.

4. The method of claim 2, wherein the spectroscopic data includes spectral data obtained from the grain sample at a plurality of different wavelengths, the method further comprising applying the spectral data as variables to the calibration equation to calculate the value.

5. The method of claim 4, wherein the calibration equation is derived by regression between spectroscopic data for non-GMO grain and GMO grain.

6. The method of claim 4, wherein the calibration equation is derived by multiple-linear regression.

7. The method of claim 4, wherein the calibration equation is derived by partial least squares.

8. The method of claim 4, wherein the calibration equation is derived by neural network analysis.

9. The method of claim 1, wherein the spectral data is near-infrared reflectance data.

10. The method of claim 1, further comprising:

applying the spectral data to a plurality of different calibration equations to calculate a plurality of values; and

determining whether the grain sample contains GMO grain based on the calculated values.

11. The method of claim 10, wherein each of the calibration equations corresponds to a calibration between a non-GMO grain and one of a plurality of different GMO grains.

12. The method of claim 11, further comprising formulating each of the calibration equations by:

subjecting a non-GMO grain to near infrared spectroscopy to generate spectroscopic data for the non-GMO grain;

subjecting a plurality of different GMO grains to near infrared spectroscopy to generate spectroscopic data for each of the GMO grains; and

analyzing differences between the spectroscopic data for the non-GMO grain relative to the spectroscopic data for each of the GMO grains to produce the calibration equations.

13. The method of claim 11, further comprising:

storing a catalog of different calibration equations in a storage device;

adding new calibration equations for newly marketed GMO grains to the catalog in the storage device; and

retrieving selected calibration equations from the storage device for use in determining whether the grain sample contains GMO grain.

14. A system for distinguishing between genetically modified (GMO) and non-GMO grain, the system comprising:

a near infrared spectroscopy device capable of producing spectral data from a grain sample; and

a processor that determines whether the grain sample contains GMO or non-GMO grain based on spectral data received from the device.

15. The system of claim 14, wherein the processor applies the spectral data to a calibration equation that defines a spectroscopic difference between a non-GMO

grain and a GMO grain to calculate a value, and determines whether the grain sample contains GMO grain based on the calculated value.

16. The system of claim 14, further comprising an indicator device connected to and capable of receiving output from the processor that provides a user with an indication of the calculated value.

17. The system of claim 15, wherein the spectral data produced by the near infrared spectroscopic device includes spectral data obtained from the grain sample at a plurality of different wavelengths, the processor applying the spectral data as variables to the calibration equation to calculate the value.

18. The system of claim 17, wherein the calibration equation is derived by regression between spectroscopic data for non-GMO grain and GMO grain.

19. The system of claim 18, wherein the calibration equation is derived by multiple-linear regression.

20. The system of claim 18, wherein the calibration equation is derived by partial least squares.

21. The system of claim 18, wherein the calibration equation is derived by neural network analysis.

22. The system of claim 14, wherein the processor is programmed to apply the spectral data to a plurality of different calibration equations to calculate a plurality of values, and determine whether the sample contains GMO grain based on the calculated values.

23. The system of claim 22, wherein each calibration equation corresponds to a calibration between non-GMO grain and one of a plurality of different GMO grains.

24. The system of claim 22, further comprising a storage device connected to the processor that stores the different calibration equations, the processor retrieving the calibration equations from the storage device for the application of the spectral data to the calibration equations.

25. A method for distinguishing between genetically modified (GMO) and non-GMO grain, the method comprising:

measuring the infrared spectral signature of a grain sample; and

determining whether the grain sample contains GMO grain based on the measurement.

26. A method for distinguishing between genetically modified (GMO) and non-GMO grain, the method comprising:

analyzing a grain sample without application of wet chemical techniques, and

determining whether the grain sample contains GMO grain based on the analysis.

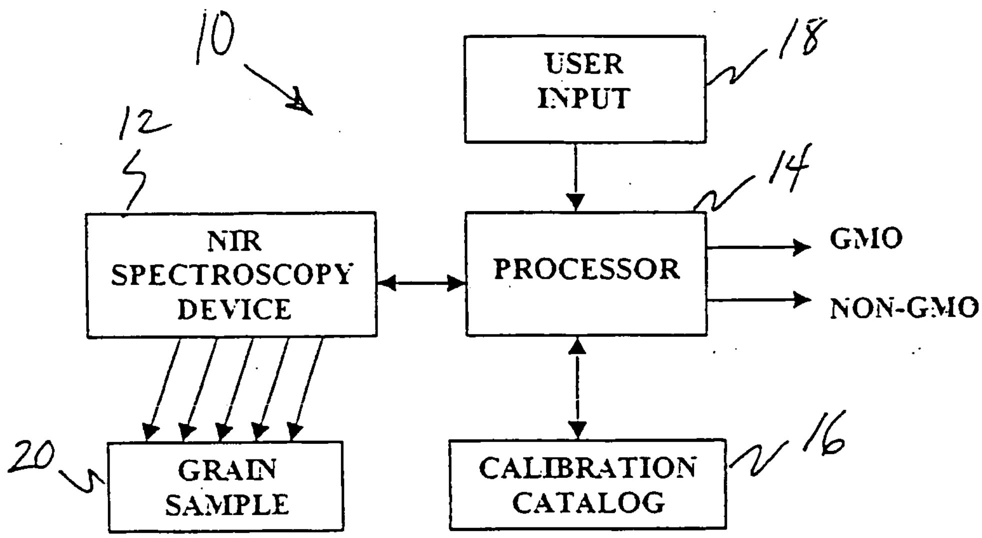
10
USER INPUT
18
12
14
NIR SPECTROSCOPY DEVICE
PROCESSOR
GMO
NON-GMO
20
GRAIN SAMPLE
CALIBRATION CATALOG
16

FIG. 1

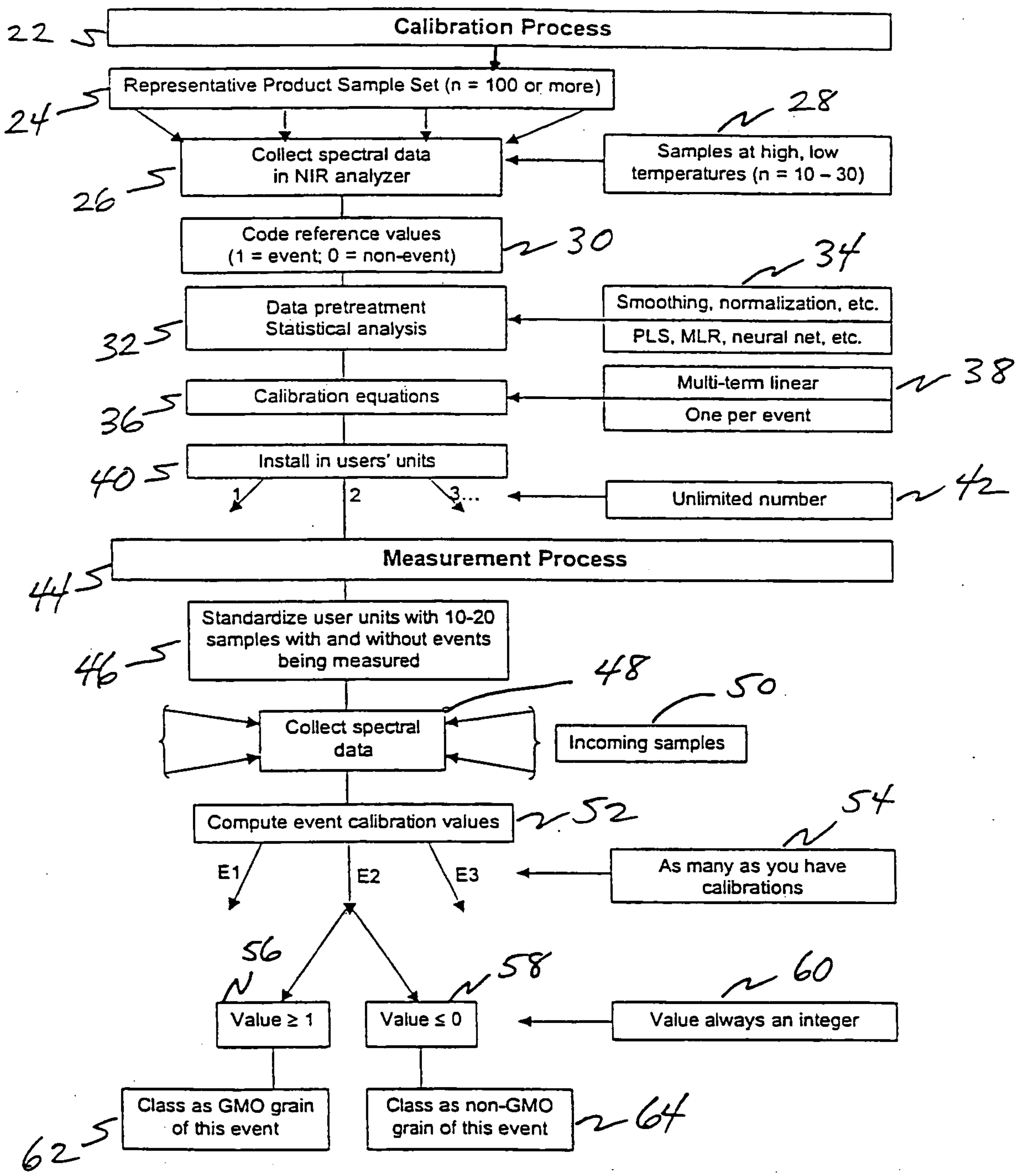
Calibration Process
Representative Product Sample Set (n = 100 or more)
Collect spectral data in NIR analyzer
Samples at high, low temperatures (n = 10 – 30)
Code reference values (1 = event; 0 = non-event)
Data pretreatment Statistical analysis
Smoothing, normalization, etc.
PLS, MLR, neural net, etc.
Calibration equations
Multi-term linear
One per event
Install in users' units
1
2
3...
Unlimited number
Measurement Process
Standardize user units with 10-20 samples with and without events being measured
Collect spectral data
Incoming samples
Compute event calibration values
E1
E2
E3
As many as you have calibrations
Value ≥ 1
Value ≤ 0
Value always an integer
Class as GMO grain of this event
Class as non-GMO grain of this event
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64

FIG. 2

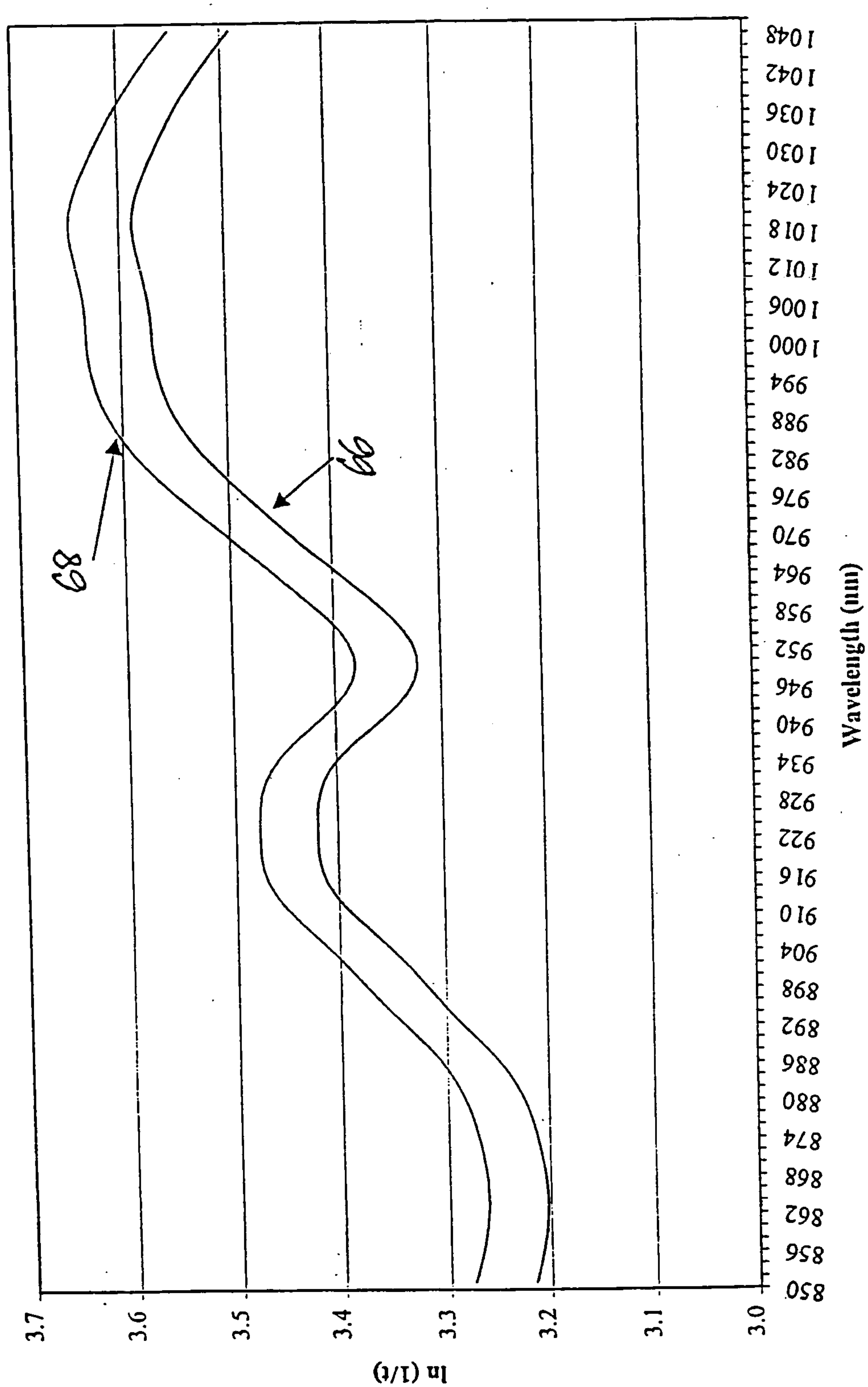
ln (1/t)
Wavelength (nm)
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
850
856
862
868
874
880
886
892
898
904
910
916
922
928
934
940
946
952
958
964
970
976
982
988
994
1 000
1 006
1 012
1 018
1 024
1 030
1 036
1 042
1 048
68
66

FIG. 3

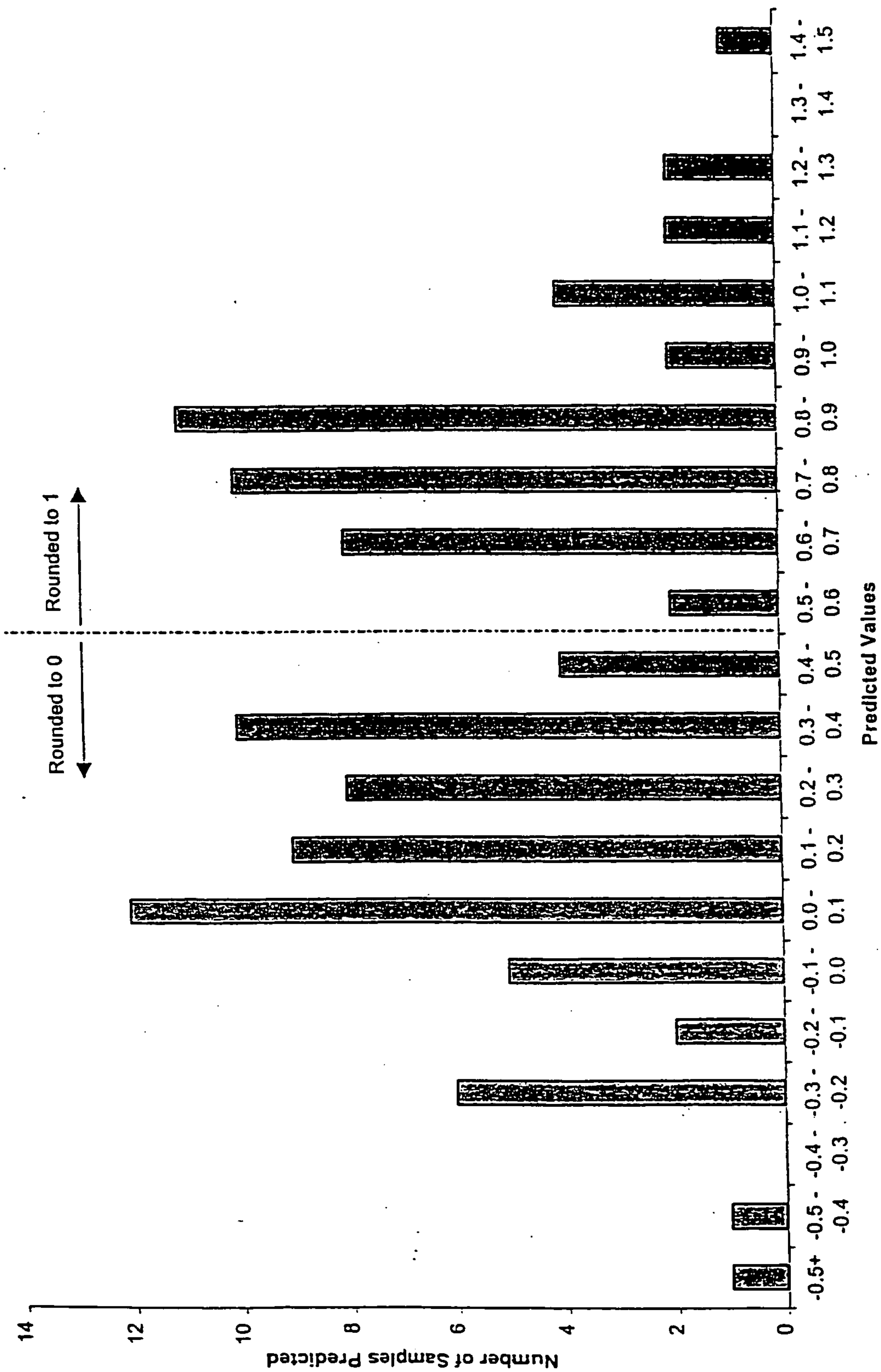
Number of Samples Predicted
0
2
4
6
8
10
12
14
Predicted Values
-0.5+
-0.5 - -0.4
-0.4 - -0.3
-0.3 - -0.2
-0.2 - -0.1
-0.1 - 0.0
0.0 - 0.1
0.1 - 0.2
0.2 - 0.3
0.3 - 0.4
0.4 - 0.5
0.5 - 0.6
0.6 - 0.7
0.7 - 0.8
0.8 - 0.9
0.9 - 1.0
1.0 - 1.1
1.1 - 1.2
1.2 - 1.3
1.3 - 1.4
1.4 - 1.5
Rounded to 0
Rounded to 1

FIG. 4

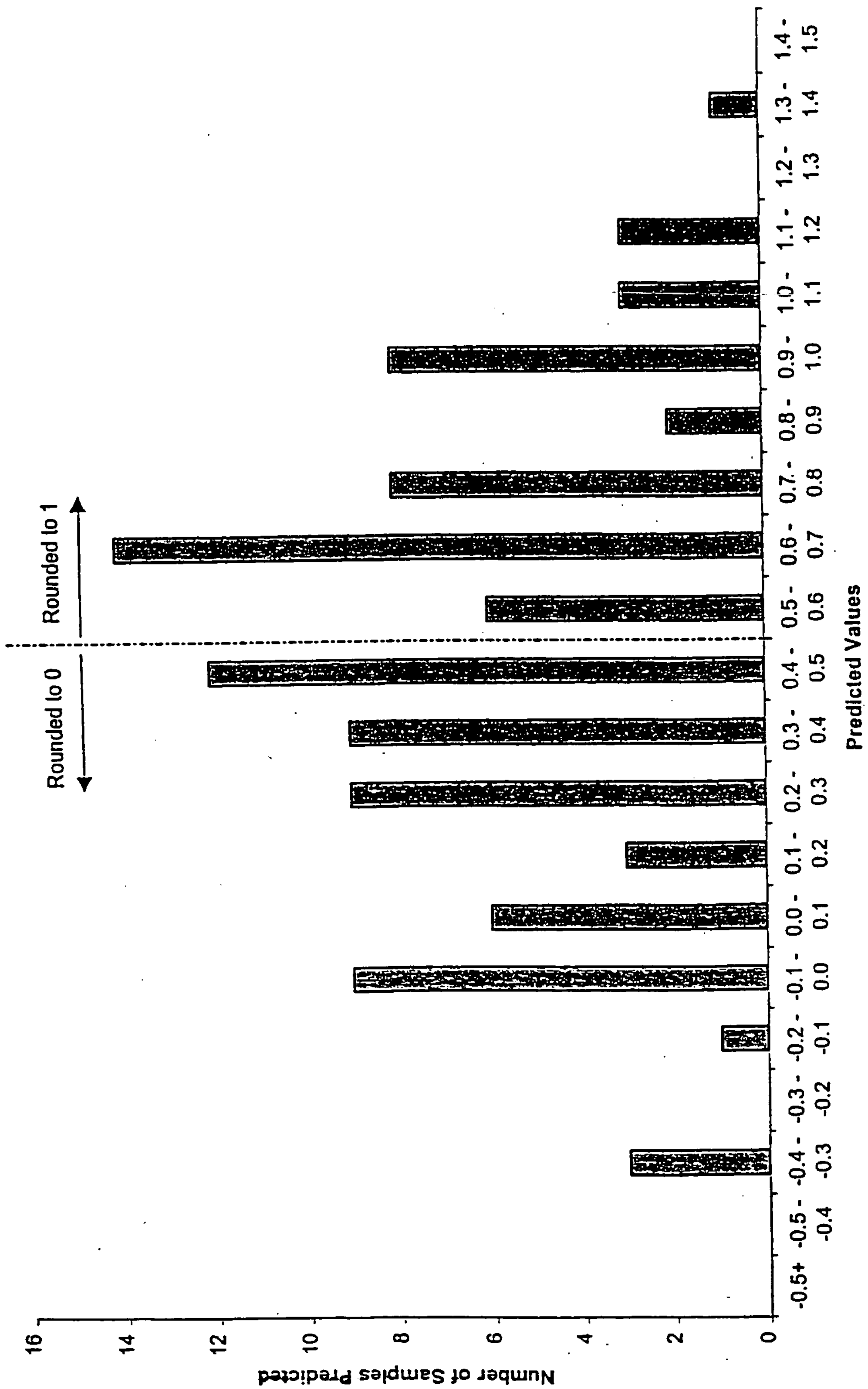
Rounded to 0
Rounded to 1
Number of Samples Predicted
0
2
4
6
8
10
12
14
16
Predicted Values
-0.5+
-0.5 - -0.4
-0.4 - -0.3
-0.3 - -0.2
-0.2 - -0.1
-0.1 - 0.0
0.0 - 0.1
0.1 - 0.2
0.2 - 0.3
0.3 - 0.4
0.4 - 0.5
0.5 - 0.6
0.6 - 0.7
0.7 - 0.8
0.8 - 0.9
0.9 - 1.0
1.0 - 1.1
1.1 - 1.2
1.2 - 1.3
1.3 - 1.4
1.4 - 1.5

FIG. 5

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/US 00/14262

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/27 G01N21/35 G01N21/25 G01N21/85 G01N21/89
A01H1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N A01H C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 44140 A (MCELROY DAVID;STEPHENS MICHAEL A; SPENCER T MICHAEL ; MUMM RITA (U) 8 October 1998 (1998-10-08)	1
Y	page 2, line 1 - line 15 page 52, line 21 - line 27 ---	2-4, 6-13,25, 26
Y	US 5 668 374 A (RANC MARTHA L ET AL) 16 September 1997 (1997-09-16) column 1, line 22 - line 24 column 2, line 5 - line 20 column 2, line 47 - line 55 column 4, line 63 -column 5, line 25 column 7, line 8 - line 13 ---	2-4,6,7, 9-13,25, 26
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2000	08/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Navas Montero, E

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 24830 A (WOLFKING DANMARK AS ;SCHWARZE HOLM (DK); JENSEN HILMER (DK); PETER) 15 August 1996 (1996-08-15) page 8, line 22 - line 25	8
X	US 5 132 538 A (NORRIS KARL) 21 July 1992 (1992-07-21) the whole document	14-26
X,P	WO 99 40419 A (DSQUARED DEV INC) 12 August 1999 (1999-08-12) page 5, line 17 -page 6, line 6 page 8, line 20 - line 31 figure 1	14-24
A	DATABASE WPI Section PQ, Week 9431, 21 September 1994 (1994-09-21) Derwent Publications Ltd., London, GB; Class P13, AN 94-253787 XP002150792 "Procedure for identifying mutant varieties of maize in genetic studies of grain -performing fluorescence of seed sections illuminated at 375-390 nanometres and establishing mutant varieties as those with reduced fluorescence." & RU 2 005 353 A (FEDENKO V S), 15 January 1994 (1994-01-15) abstract	1,14,25, 26
A	DATABASE WPI Section Ch, Week 9431, 15 January 1994 (1994-01-15) Derwent Publications Ltd., London, GB; Class C07, AN 94-253786 XP002052054 FEDENKO V S; GLUSHKO V V; STRUZHKO V S.: "Determination of specific tendency to accumulate carotenoids -in endosperm mutants of maize, involves measuring optical density of grain sample reflection at specified spectrum range." & RU 2 005 352 A (FEDENKO V S), 15 January 1994 (1994-01-15) abstract	1

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Interna   al Application No
PCT/US 00/14262

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9844140 A	08-10-1998	US 6040497 A	21-03-2000
		AU 6882298 A	22-10-1998
		BR 9808475 A	23-05-2000
		EP 0975778 A	02-02-2000
US 5668374 A	16-09-1997	NONE	
WO 9624830 A	15-08-1996	DK 15595 A	01-07-1996
		DK 9196 A	11-08-1996
		AU 4619396 A	27-08-1996
		AU 4619496 A	27-08-1996
		AU 4619596 A	27-08-1996
		CA 2212626 A	15-08-1996
		CA 2212700 A	15-08-1996
		DK 9096 A	11-08-1996
		WO 9624843 A	15-08-1996
		WO 9624835 A	15-08-1996
		EP 0871878 A	21-10-1998
		EP 0808451 A	26-11-1997
		US 5918977 A	06-07-1999
		US 6020588 A	01-02-2000
US 5132538 A	21-07-1992	CA 2067526 A	25-11-1992
WO 9940419 A	12-08-1999	US 6100526 A	08-08-2000
		AU 6659098 A	23-08-1999
RU 2005353 A	15-01-1994	NONE	
RU 2005352 A	15-01-1994	NONE	

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Date: 03/01/2003